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Extended Summaries Pesticides in Food and Drink

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Integrated Pest and Disease Control and Pesticide Residues: Results of a Four-Year Study

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Introduction

Of the many techniques adopted to reduce the impact of pesticide residues in vegetables and fruit, good agricultural practice and integrated pest management practice (IPM) are preferred. The main reason for this is that both respect the environment and the farm economy.

In IPM, pesticide application is carried out only when the pest damage has reached a significant level; the pesticide selected has to be used in accordance with the National and European directives and must have low toxicity towards man and low persistence in the environment. The reduction of pesticide used, implicit in this strategy, respects the environment and human health and also farm productivity. However its results and efficiency depend essentially upon the professional approach of the operators and upon the weather.

In recent years, much attention has been paid to the use of IPM, but no field monitoring has been carried out in Italy to investigate the effect of IPM on residue levels in the human diet. However, in Italy two major monitoring plans have been carried out recently: the National Monitoring coordinate by the Agriculture Ministry in 1994,[1] which analysed fruit and vegetables collected directly during harvest in farms in the north of Italy and the National Monitoring coordinate by the Health Ministry[2] which analysed pears collected in wholesale and retail markets.

The work reported here is part of a project which aims to evaluate the effects of IPM on pesticide residues in vegetable and fruit. The network includes the Catholic University (ICAA) and two local farm organisations the CO.DI.MA (COnsorzio DIfesa MAntova) and the Consorzio Pera Tipica Mantovana. Since 1992 residues in produce from 45 farms have been analysed in the fruit and vegetables after harvest. Table 1 reports the main pesticides analysed. The data reported here concern pear crops.

Experimental plan

The fungicides dithiocarbamates, captan, dichlofluanid and procymidone and the insecticides quinalphos, azinphos-methyl, carbaryl and chlorpyrifos-methyl were chosen for this study because they were used successfully in the farms for the whole four years, and almost all samples showed residues greater than the Limit Of Detection (LOD).

All the farms chosen were monitored strictly for four years under the supervision of specialised personnel who recorded all the relevant agronomic information (e.g. characteristics of the treatment, climatic data, etc.). Some of the information collected is reported in Table 2. The farms are all located in Mantova Province on the right-hand side of the Po river, an area with a common climatic pattern (Table 2). The site is well-cropped with pears (*Pyrus domestica* Medic.), the main cultivars being

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TABLE 1
Pesticides Monitored in All Years (1992–1995) in Pear Samples

Pesticide	*No. of analyses*	*MRL* (*mg kg*$^{-1}$)	*>MRL* (%)
Azinphos-methyl	205	0·5	1·1
Captan	205	3	0
Carbaryl	205	3	1·0
Chlorothalonil	119	0·3	0
Chlorpyrifos	205	0·2	0
Chlorpyrifos-methyl	205	0·2	0
Demeton-*S*-methyl	137	0·4	0
Dichlofluanid	205	5	0
Dithiocarbamates	205	2	0·5
Iprodione	80	5	0
Miclobutanil	98	0·2	0
Parathion	182	0·5	0
Phosalone	182	2	0
Procymidone	205	1·5	0
Quinalphos	205	0·1	0
Vamidothion	182	0·5	0

[a] Italian Maximum Residue Level (Ref. 3).

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Sampling

Representative samples of fruit were collected during harvest from each farm and for each cultivar. Sampling was performed following good agricultural practice and the guidelines for the measurement of the maximum residue level as required by Italian law.[3,4] The operator collected one fruit at random from each filled container (30 kg) or five pears in the case of big containers (400 kg). In total 50–60 pears were collected for each cultivar from each farm.

All the sampled pears were divided into four pieces, mixed, and a representative sample of 2 kg was stored at −20°C until analysis.

Extraction and analysis

Pesticide residue analyses were carried out using published methods[5,6] and the Italian official method for dithiocarbamates.[7]

The method of Dagna *et al.*[5] is based on extraction of pesticides from homogeneous sample pulp sorbed onto diatomaceous earth to obtain a free-flowing powder, which is extracted in a glass column with dichloromethane. Recovery studies were performed at 0·2, 1 and 5 mg kg^{-1} levels and recoveries of all pesticides were always better than 80%.

Dithiocarbamate analyses were performed by transformation into carbon disulfide and colorimetric determination and recoveries were also better than 80%.

In extracted samples chlorpyrifos-methyl, quinalphos and azinphos methyl were detected by GC/NPD; captan, procymidone and dichlofluanid by GC/ECD and carbaryl by HPLC/DAD. All samples giving positive results were confirmed by GC/MS.

Chromatographic conditions were as follows:

—GC/NPD detection. GC Dani 8510 equipped with NPD detector and DB-1 fused-silica column, 30 m × 0.23 mm ID, 0·25 μm film thickness (J&W Scientific, Folsom, CA 95630); operating conditions: injector 260°C; detectors 280°C; temperature program, column 140°C, hold 3 min, increase to 200°C at 2°C min^{-1}, hold 1 min, increase to 240°C at 10°C min^{-1}, hold time 10 min; helium carrier gas (5 ml min^{-1}), H_2 working gas (2·4 ml min^{-1}), N_2 make-up (25 ml min^{-1}); air 190 ml min^{-1}; split 30 ml min^{-1}; injection volume: 1 μl.

—GC/ECD detection. GC Dani 8521 equipped with ECD detector and DB-17 fused-silica column, 30 m × 0·25 mm ID, 0·25 μm film thickness (J&W Scientific); operating conditions: injector 280°C; detectors 300°C; temperature program, 190°C, hold 2 min, increase to 240°C at 3°C min^{-1}, hold time 10 min; helium carrier gas (7·5 ml min^{-1}), N_2 make-up (25 ml min^{-1}), split 30 ml min^{-1}; injection volume: 1 μl.

—HPLC/DAD detection. Hewlett Packard 1090 L liquid chromatograph with diode array detector set at

TABLE 2
Main Climatic Characteristics of the Area

Year	*Temperature (°C)*		*Rain* (*mm*)	*Rainy days*	*DT25-30*[a] (°*C*)	*Air relative humidity* (%)
	Average	*Cumulative*				
1992	13·7	5009·3	359·8	41	45	70·2
1993	12·0	4408·7	608·8	65	21	72·8
1994	13·5	4939·7	411·9	50	35	70·2
1995	12·1	4422·2	459·1	56	30	71·1

[a] Number of days with mean daily temperature between 25 and 30°C.

240 nm equipped with RP 18 Merck 5 μm column (12·5 cm × 4 mm ID), mobile phase acetonitrile + water (65 + 35 by volume), flow rate 1 ml min^{-1}; injection volume: 50 μl.

—GC/MS confirmation. GC Varian with MS ITS40 Finnigan Mat and DB-5 fused-silica column 30 m × 0·25 mm ID, 0·25 μm film thickness (J&W Scientific); operating conditions: injector 260°C; transfer line 260°C; temperature program, 160°C, hold 5 min, increase to 240°C at 5°C min^{-1}, hold time 260°C; temperature program, 160°C, hold 5 min, increase to 240°C at 5°C min^{-1}, hold time 20 min; helium carrier gas (60 ml min^{-1}); injection volume: 0·5 μl; mass range 50–400 amu, 1 second per scan.

Detection limits are reported in Table 3.

Statistical analysis

All residue data were analysed statistically using the SAS programme. First, correlation coefficients were determined between classified variables (number of treatments, time between the last treatment and harvest, estimated dose applied, total rain, number of rain events, cumulated temperature, humidity, days with temperature ranging between 25 and 30°C, yearly temperature) to identify those variables that were not co-linear such as the number of treatments, the time between the last treatment and harvest and cumulated temperature. Then ANOVA and multiple regressions were carried out using the non-colinear variables.

When residues of samples were lower than limits of detection (LOD), they were considered to be zero. Any variation in residue levels between cultivars was not evaluated.

Results and discussion

The pesticide residues measured (mean and range concentration) are reported in Table 3. Residues in a large proportion of the samples were below the limit of detection and only 23 samples out of 205 had concentrations >50% of the MRL (Maximum Residue Limit). Only four out of 205 samples analysed had residues >MRL, these being azinphos-methyl, carbaryl and dithiocarbamates.

Residues of dithiocarbamates, carbaryl, azinphos-methyl and procymidone were greater than those of the other pesticides tested. This could be explained on the basis of the agronomic practices and climatic conditions during the experiment. In fact, statistical analysis showed that three main parameters could affect this behaviour, i.e. number of treatments, time between the last treatment and harvest and the cumulated temperature. Carbaryl and azinphos-methyl residues are not well described by the statistical model adopted while results for captan, chlorpyrifos-methyl, dichlofluanid and procymidone were dependent on the rank variables of the model, with an F probability > 0·001 (Table 4).

Increasing the number of treatments resulted in an increase in the level of procymidone, quinalphos and chlorpyrifos-methyl residue concentration ($t > 0·001$), while decreasing the time between the last treatment and the harvest resulted in a significant increase only for captan. The cumulated temperature seemed to reduce the residue level only for dichlofluanid; a different behaviour was showed by captan which cannot be explained.

The comparison between our data and those of the Agriculture Ministry, shows that the percentage of our positive samples is always lower (Table 5) except with carbaryl residues, probably due to the smaller number of samples analysed by the Ministry of Agriculture (79 compared to 205). Furthermore, the comparison shows evidence that, for all pesticides, the number of samples with residue level higher than LOD is small in both cases. Data obtained by the Health Ministry show lower residue levels compared with the other two sources (Table 5). This could be explained on the basis that samples used in that monitoring study were taken in the market and not from the farms, which means that

TABLE 3
Summary of the Analytical Results Obtained in the Four Years

Pesticide	*Detection Limit* (*mg kg*$^{-1}$)	*Range* (*mg kg*$^{-1}$)	*Mean* (± *standard deviation*) (*mg kg*$^{-1}$)
Dithiocarbamates	0·1	0–3	0·4 (±0.3)
Carbaryl	0·01	0–3·34	0·16 (±0·44)
Azinphos-methyl	0·07	0–1·29	0·04 (±0·14)
Chlorpyrifos-methyl	0·007	0–0·08	0·003 (±0·012)
Captan	0·1	0–0·3	0·1 (±0·1)
Dichlofluanid	0·02	0–0·32	0·04 (±0·08)
Procymidone	0·02	0–1·39	0·06 (±0·15)
Quinalphos	0·008	0–0·08	0·005 (±0·016)

TABLE 4
Results of the Regression Analyses (CONCRES) and Number of Treatments (NT), Days between the Last Treatment and the Harvest (DTH) and the Cumulated Yearly Temperature (TCUM)[a]

Pesticide	$p \geqslant t$			$p > F$
	NT	*DTH*	*TCUM*	
Azinphos-methyl	+0·0603	−0·7936	−0·1359	0·0615
Captan	+0·7296	−0·0057**	+0·0026**	0·0001***
Carbaryl	−0·5277	−0·2012	−0·5474	0·5058
Chlorpyrifos-methyl	+0·0036**	−0·0317*	−0·9722	0·0001***
Dichlofluanid	+0·0120*	−0·0143*	−0·0029**	0·0001***
Dithiocarbamates	+0·0381*	−0·0537	−0·1405	0·0004**
Procymidone	+0·0001***	−0·2941	−0·4062	0·0001***
Quinalphos	+0·0003***	−0·3233	−0·4852	0·0007***

[a] ***; **; * probability higher than F or t at 0·001, 0·005 and 0·05 respectively.

the time elapsed from the last treatment and the analysis was greater.

The residues of dithiocarbamates measured, comprising mainly thiram, ziram and mancozeb, methiram and propineb, correlated positively with the number of treatments, as reported in Table 4. On average, this number of treatments is still high (12·1 treatments per year) although compared with the traditional pest control schedules, IPM involves *c.*20% fewer treatments. However, in spite of the high use of these pesticides, only one of the analysed samples had a residue >MRL. This sample had received the highest number of treatments and the lowest number of days between the last treatment and harvest (11 days). Climatic conditions had no effect on the residues level. The dithiocarbamates very often fell in the class >10% MRL (59 of the samples) and rarely in the class >50% MRL (2.4%) (Table 5).

The average number of treatments with procymidone was constant at 1·3 per year and the time between the last treatment and harvest was constant, with the exception of 1993 when it was 50 days. No samples taken during the four years had residues >MRL and only one was >50% MRL. Residues of 13% of the samples were >10% MRL (MRL for procymidone is 0·15 mg kg^{-1}).

The position with quinalphos was different. The number of treatments per year decreased during the study and averaged 0.3. The interval between the last treatment and harvest was *c.*50 days. In the first year some samples had residues >50% MRL because the harvest interval (DTH) was 20–25 days: this practice was changed in the following year, resulting in reduction of the residues (between 1992 and 1995 all residues were below the LOD).

During the study, azinphos-methyl was applied 1·3 times on average per year with an interval of *c.*60 days between the last treatment and harvest (it varied from 76 days in 1994 to 46 days in 1995). For all samples in 1994–1995 the residues were below the LOD. In 1993

TABLE 5
Pesticide Residues in Pear Fruits Measured in the Four Years by the Three Different Organisations. The Values are Expressed as Percentage of the Legal Maximum Residue Level (MRL).

Pesticide	*ICAA*				*Agric Ministry*[a]			*Health Ministry*[a]			
	No.	>10%[b]	>50%[b]	>*MRL*	*No.*	>10%[b]	>50%[b]	*No.*	>10%[b]	>50%[b]	>*MRL*
Azinphos-methyl	205	9·3	3·9	1·0	1223	7·8	2·4	301	18·9	3·7	0·0
Captan	205	8·3	1·0	0·0	160	1·2	0·0	256	1·6	0·0	0·0
Carbaryl	205	7·3	0·5	0·5	79	0·0	0·0	305	1·3	0·7	0·3
Chlorpyrifos-methyl	205	4·4	0·0	0·0	590	1·0	1·2	263	1·5	0·0	0·0
Dichlofluanid	205	0·0	0·0	0·0	229	0·0	0·0	281	3·6	0·0	0·0
Dithiocarbamates	205	59·0	2·4	0·5	1174	9·1	2·6	316	12·0	17·1	0·0
Procymidone	205	8·8	0·5	0·0	350	2·6	0·3	348	8·9	1·1	0·0
Quinalphos	205	6·3	2·9	0·0	214	5·1	2·3	255	20·8	2·4	0·0

[a] National monitoring carried out by Agricultural Ministry[1] and the Health Ministry.[2]
[b] Percentage of the MRL. These classes are used by some market organisations and cooperative of consumers to define the quality of the food.[8]

two samples were >MRL and both were collected from the same farm which used short DTH (47 and 30 days).

Captan was applied on average 0·6 times per year. IPM resulted in a reduction of the number of treatments (NT) per year which dropped from a mean of 1·2 to 0·5 times over the four-year period. The DTH was the same for the whole period (70 days). Decreasing the number of treatments also decreased the number of samples with residues >10% MRL (from 17 in 1992 to 2 in 1993, to zero in 1994–1995).

Treatments with carbaryl remained constant for each farm and averaged 0·5 per year, with a DTH of 18 days. Samples with residues >LOD were found in *c.*7% of the total samples and were often in the >10% MRL class, but in 1993 a few samples were in >50% MRL class.

Dichlofluanid residues increased between 1992 and 1995, whereas the interval between the last treatment and harvest decreased proportionally from 33 to 13 days. However, with the high MRL of 5 mg kg^{-1} all the samples had residues <10% MRL.

The number of samples with residues of chlorpyrifos-methyl seemed to increase over the period of the study, probably because the number of treatments with this active ingredient increased from a mean of 0·3 to 1·0. All samples containing residues were in the >10% MRL class.

The residue data were compared with toxicological data related to the human diet, using the guidelines indicated in UNEP/FAO/WHO Food contamination.[8] The objective was to estimate the daily pesticide residue intake from pears eaten by consumers. This Estimated Daily Intake of residues (EDI) is calculated from the average consumption per person of pears,[9,10] reduced by a factor of 0·7 to account for the residues lost as a result of factory transformation into juice. Table 6 shows the comparison between EDI and the Acceptable Daily Intake (ADI) of residues for our data (1992–1995) and those of Camoni *et al.*[11] for 1990–1991.

Both studies show that the percentage of ADI ingested with the pears is very low being <0·5% for each pesticide with the total intake never exceeding 1·5% of the ADI. The adoption of IPM results in reduction of the ADI ingested except for dithiocarbamates and for chlorpyriphos-methyl. For dithiocarbamates we can presume that it is the result of the high number of treatments with this compound which are essential for the control of the fungus (*Stemphylium vesicaria* (Wallr.)Simmons), which is a particular problem in this area of Italy so that the use of the dithiocarbamates is very high compared with that in other Italian areas.

Conclusions

This study shows that IPM is a useful technique for reducing the amount of pesticide residues in pear fruit. Particular attention needs to be given to the number of treatments and to the time between the last treatment and harvest, there being a legal minimum interval in Italy.

The strategy of reducing the number of treatments and increasing DTH is not possible with dithiocarbamates in the area studied because proper control of *S. vesicaria* requires more treatments and therefore results in the frequent occurrence of residues of this class of pesticide in fruit. The analytical method used to measure dithiocarbamate residues (measurement of total carbon disulfide liberated) does not help with determining the effect of individual dithiocarbamates on the total residue measured or with

TABLE 6
Comparison between the Acceptable Daily Intake (ADI) and the Estimated Daily Intake (EDI)[a] of Pesticide Residues in Pear Fruits derived from Present Study (ICAA) and Those carried out by Italian Health Ministry in 1990–1991

		Health Ministry		*ICAA*	
Pesticide	*ADI* ($\mu g\ kg^{-1}\ body\ wt\ day^{-1}$)	*EDI* ($\mu g\ kg^{-1}\ body\ wt\ day^{-1}$)	*ADI ing.* (%)	*EDI* ($\mu g\ kg^{-1}\ body\ wt\ day^{-1}$)	*ADI ing.* (%)
Azinphos-methyl	5	0·0186	0·37	0·0162	0·32
Captan	100	0·1784	0·18	0·0721	0·07
Carbaryl	10	0·0360	0·36	0·0374	0·37
Chlorpyrifos-methyl	1	0·0003	0·03	0·0014	0·14
Dichlofluanid	300	0·0255	0·01	0·0180	0·01
Dithiocarbamate[b]	50	0·1204	0·24	0·2603	0·52
Procymidone	100	0·0842	0·08	0·0284	0·03
Quinalphos[c]	15	—	—	0·0023	0·01
Total			1·27		1·44

[a] EDI is calculated according to Camoni *et al.*[12] and as ADI = yearly intake residues/365·6.
[b] As mancozeb.
[c] Data not published.

optimisation of the use of these compounds in practice. Alternatives to dithiocarbamates are procymidone and copper, and changes in agronomic practice (such as reducing irrigation and fertilisation, reduction in soil water content, etc.): the alternative fungicides are not better than dithiocarbamates against the pest because they have no systemic activity, and the agronomic changes are not always feasible.

There is the evidence that by following good agricultural practice, which is included in the IPM programme, the Maximum Residue Level of the pesticides studied in pear fruit is never exceeded. The exception is with azinphos-methyl. To avoid the risk of having their products withdrawn from the market, it is important to decide whether it is necessary to revise the value for this compound which is cited in the OMS.[3]

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